Plant Physiol. (1992) 98, 392–394
0032-0889/92/98/0392/03/$01.00/0

Received for publication August 8, 1991
Accepted September 12, 1991

*Communication*

# Occurrence of Temperature-Sensitive Phenotypic Plasticity in Chlorophyll-Deficient Mutants of *Arabidopsis thaliana*[1]

**John Markwell* and John C. Osterman**

*Department of Biochemistry (J.M.) and School of Biological Sciences (J.C.O.), University of Nebraska, Lincoln, Nebraska 68583-0718*

## ABSTRACT

**A collection of 75 putative mutants with alterations in leaf pigmentation was visually selected from *Arabidopsis thaliana* plants ($M_2$ generation) grown at 26°C from seeds treated with the mutagen ethylmethanesulfonate. Fifty-eight of the plants were found to have chlorophyll contents decreased by at least 10% from the parental Columbia ecotype. These plants were screened for chlorophyll content and the ratio of chlorophyll *b*/*a* after growth at 20 or 26°C. Relative to the parental type, a significant number of individuals in which the chlorophyll-deficient phenotype was exacerbated at one of the growth temperatures were identified. We conclude that temperature-sensitive phenotypic plasticity for chlorophyll content is relatively common in mutant populations of higher plants.**

To elucidate the interrelationship of chloroplast thylakoid membrane structure, composition, and function, many studies have been conducted on Chl-deficient mutants of higher plants (13, 17). As in other areas of biochemistry, mutants can provide valuable systems for examining the functioning of a normal organism. Such Chl-deficient mutants have been described in barley (6), maize (7, 11) and sweetclover (3, 15, 19), among other species. However, few of these mutants have been characterized with regard to their specific biochemical defect. Although most such mutants have been grown under only one set of environmental conditions, a number of these mutants exhibit phenotypic plasticity in response to environmental variables such as temperature (4, 8, 9, 12, 16), photoperiod (1, 5, 9), and PPFD (7, 11).

For the past few years, a portion of our work has focused on individuals from a collection of sweetclover (*Melilotus alba*) mutants (3, 15, 19) isolated by Drs. H.J. Gorz (USDA-ARS) and F.A. Haskins (Department of Agronomy, University of Nebraska). The plants examined consist of Chl-deficient mutations falling into eight different nuclear complementation groups (3, 15, 19). One of these mutants lacks Chl *b* (the *ch5* mutant), whereas the others contain detectable amounts of this pigment. We have previously reported (20) that six of the individuals in this collection are markedly temperature-sensitive for Chl expression when grown in the temperature range of 17 to 26°C. To determine if temperature-sensitive individuals are as common among Chl-deficient mutants of higher plants as our previous study suggested (20), we produced a population of putative Chl-deficient mutants of *Arabidopsis thaliana* and screened the individuals for Chl expression at two different temperatures. Our results indicate that temperature-sensitive mutants of higher plants can be readily obtained during screenings of plants grown from mutagenized seeds.

## MATERIALS AND METHODS

Seed ($M_2$ generation) produced by *Arabidopsis thaliana* (L.) Heynh. (ecotype Columbia) plants grown from seed treated with the mutagen ethylmethanesulfonate (18) was the generous gift of Dr. C. Donald Miles. Two grams of seed, equivalent to approximately 100,000 seeds (18), was planted on a commercial soil-vermiculite mixture, incubated in a growth chamber at 26°C, 60% RH, 16 h photoperiod, and a PPFD of 250 to 400 $\mu$mol $m^{-2}$ $s^{-1}$, and supplemented weekly with a mineral salts solution (10). As the plants grew, individuals that subjectively appeared to have leaves of a lower Chl content than the normal *A. thaliana* Columbia ecotype were removed from the flats and grown in separate pots until seeds could be collected.

Seeds from these putative Chl-deficient mutants were planted and grown in chambers under the above conditions except that a duplicate set was grown at 20°C. Leaves from plants grown at both temperatures were excised, usually when plants reached the five-leaf stage, and analyzed for Chl content (14). Chl contents are the result of three to five determinations. The data appeared to be independent of the particular leaf chosen from a plant for analysis. None of the putative mutants reported herein appear to be of the slow-greening or virescent phenotype. The putative mutants were also grown in polycarbonate boxes on a mineral salt medium (10) containing 1% (w/v) sucrose and 0.82% (w/v) agar at both 20 and 26°C to identify any mutant lines that segregated into both normal and pigment-altered individuals. To date, we have completed analyses on 75 nonsegregating putative mutant isolates.

[1] This work was supported by grants GM89-00641 of the U.S. Department of Agriculture and DMB87-03100 of the National Science Foundation and by the University of Nebraska-Lincoln Center for Biotechnology and Research Council. Paper No. 9670, Nebraska Agricultural Research Division.

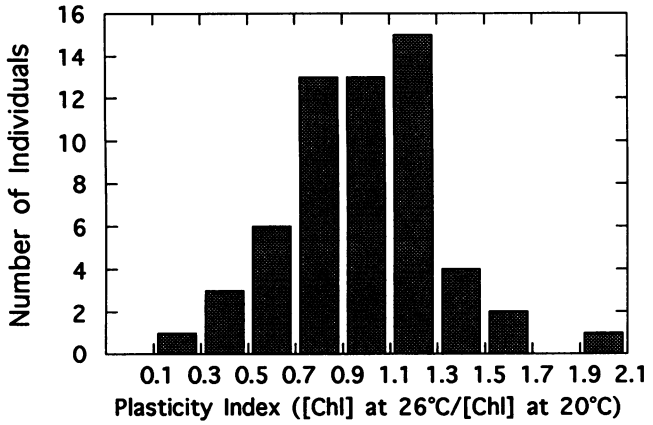


**Figure 1.** A plot demonstrating the distribution of the plasticity index for each of the 58 *A. thaliana* putative mutants.

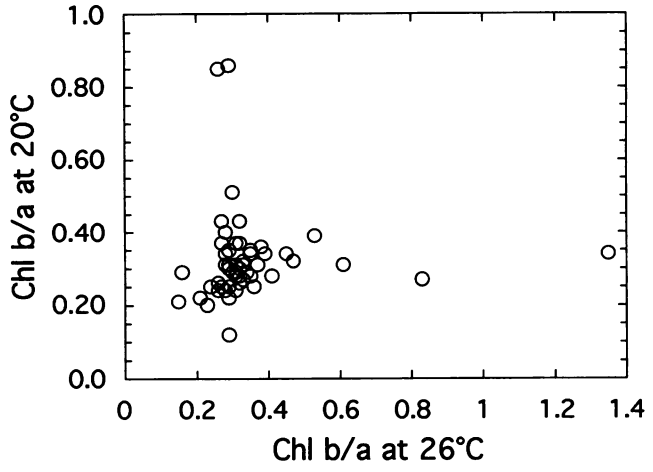


**Figure 3.** A scatter plot demonstrating the temperature sensitivity of Chl *b/a* ratios for 58 putative mutants of *A. thaliana* grown at 26 or 20°C.

## RESULTS AND DISCUSSION

From 75 *A. thaliana* putative mutants selected visually on the basis of leaf coloration, 58 had ≤90% of the Chl content of the Columbia ecotype when grown at 26°C. These individuals were further examined. As a measure of phenotypic plasticity in response to growth temperature (termed the plasticity index), the ratio of $[Chl]_{26°C}/[Chl]_{20°C}$ was calculated. Previous studies with sweetclover mutants (20) utilized a 9°C difference (17 *versus* 26°C), whereas the plasticity index for the *A. thaliana* mutants was measured over a 6°C difference in growth temperature. The temperatures in the present study were chosen to give more rapid and less stressed growth of the *Arabidopsis* plants, because they did not grow as rapidly at 17°C as the sweetclover plants. The mean (and SE) for 20 determinations of Chl for the Columbia parental type grown at 20 and 26°C were 1.71 ± 0.04 and 2.07 ± 0.03 $\mu$mol $g^{-1}$, respectively, which is a plasticity index of 1.21 (*i.e.* 2.07/1.71 = 1.21). The distribution of the plasticity index among the selected putative mutant population is presented in Figure 1. Compared with the plasticity index for the Columbia ecotype, there is a significant amount of divergence from 1.21 among the putative mutants; over half of the population has values outside of the 1.0 to 1.4 range. These data demonstrate the varied pigment content of the selected individuals and substantiate previous results with mutants of sweetclover (20), thus demonstrating that phenotypic plasticity can involve expression of greater pigment levels at either the higher or lower of the two growth temperatures. A comparison of the Chl content of leaves of the putative mutants by scatter plot analysis (Fig. 2) indicates that the degree of phenotypic plasticity in response to growth temperature within the range of 20 to 26°C is not strictly correlated with the Chl content at the growth temperature used for the initial screening.

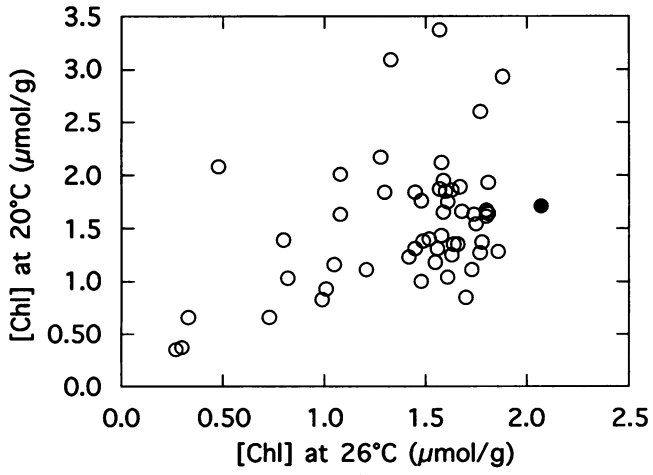


**Figure 2.** A scatter plot demonstrating the phenotypic plasticity for Chl production of 58 putative mutants of *A. thaliana*. The ratio of Chl content at 26°C is plotted against the Chl content at 20°C. Symbols: (○) putative mutant; (●) Columbia ecotype.

Many Chl-deficient mutants of higher plants are also characterized by abnormal ratios of the two primary leaf pigments, Chl *a* and Chl *b*, with many mutants being relatively more deficient in the latter pigment. Whereas the relative abundance of these two pigments is usually expressed as the Chl *a/b* ratio, this value is sensitive to errors in the estimation of Chl *b*, the lesser abundant of the two pigments, and is normally accurate only for a comparatively narrow range of values (2). We have found that the ratio of Chl *b/a* is less susceptible to such errors, approaching zero, rather than infinity, as the Chl *b* content decreases. The Chl *b/a* ratio for the Columbia ecotype was found to be 0.34 in plants grown both at 20 and 26°C. The Chl *b/a* values for the putative mutants grown at 20 or 26°C are shown in Figure 3. Although the values for the putative mutants cluster around that of the Columbia ecotype at both growth temperatures, approximately one-quarter of the individuals show significantly different values for at least one of the growth temperatures. It is interesting to note that many of the putative mutants with abnormal ratios of Chl *b/a* show much greater variation from normal at one of the two temperatures, consistent with a temperature-sensitive plasticity for the relative abundance of the two Chl species as well as for total Chl content.

In the two cases that we have examined, a characterized collection of eight nonallelic Chl-deficient mutants of sweetclover (20) and an as yet uncharacterized group of 58 putative mutants of *A. thaliana* with at least 10% alteration in Chl content described herein, it is clear that a sizable proportion of the individuals are temperature-sensitive for Chl expression. There are many poorly understood steps in the biochemistry of Chl biosynthesis and in the regulation and assembly of the photosynthetic apparatus for which mutants would be valuable tools. Unfortunately, such mutants are often lethal. As with studies on microbes, the availability of temperature-sensitive mutants could be valuable for complementing biochemical analyses on problems such as Chl biosynthesis. The heretofore underutilization of such mutants is probably attributed to growth of most mutants in the absence of strict temperature control, or the use of differential day/night temperatures. We conclude that temperature-sensitive mutants with a Chl-deficient phenotype are readily obtained and can play a valuable role in studies on porphyrin biosynthesis. It has been observed that a marked temperature modulation of plant height can also be seen in a number of dwarf mutants (M. Bevins, J. Markwell, unpublished), and we feel that temperature sensitivity will prove to be common in mutants affected in pathways other than Chl biosynthesis.

## LITERATURE CITED


1. **Allen KD, Duysen ME, Staehelin LA** (1988) Biogenesis of thylakoid membranes is controlled by light intensity in the conditional chlorophyll *b*-deficient *CD3* mutant of wheat. J Cell Biol **107:** 907–919
2. **Boardman NK, Thorne SW** (1971) Sensitive fluorescence method for the determination of chlorophyll *a*/chlorophyll *b* ratio. Biochim Biophys Acta **253:** 222–231
3. **Gengenbach BG, Gorz HJ, Haskins FA** (1970) Genetic studies of induced mutants in *Melilotus alba*. II. Inheritance and complementation of chlorophyll-deficient mutants. Crop Sci **10:** 154–156
4. **Ghirardi ML, Melis A** (1988) Chlorophyll *b* deficiency in soybean mutants. I. Effects on photosystem stoichiometry and chlorophyll antenna size. Biochim Biophys Acta **932:** 130–137
5. **Greene BA, Allred DR, Morishige DT, Staehelin LA** (1988) Hierarchical response of light harvesting chlorophyll-proteins in a light-sensitive chlorophyll *b*-deficient mutant of maize. Plant Physiol **87:** 357–364
6. **Highkin HR** (1950) Chlorophyll studies on barley mutants. Plant Physiol **25:** 294–306
7. **Hopkins WG, Hayden DB, Neuffer MG** (1980) A light sensitive mutant in maize (*Zea mays*). I. Chlorophyll, chlorophyll-protein and ultrastructural studies. Z Pflanzenphysiol **99:** 417–426
8. **Markwell JP, Chelgren TS** (1988) Chlorophyll expression varies with developmental state in the temperature-sensitive *ch4* mutation of *Melilotus alba*. Plant Physiol **87:** 172–175
9. **Markwell JP, Danko SJ, Bauwe H, Osterman J, Gorz HJ, Haskins FA** (1986) A temperature-sensitive chlorophyll *b*-deficient mutant of sweetclover (*Melilotus alba*). Plant Physiol **81:** 329–334
10. **Miksche JP, Brown JAM** (1965) Development of vegetative and floral meristems of *Arabidopsis thaliana*. Am J Bot **52:** 533–557
11. **Miles CD, Markwell JP, Thornber JP** (1979) Effect of nuclear mutation in maize on photosynthetic activity and content of chlorophyll-protein complexes. Plant Physiol **64:** 690–694
12. **Millerd A, McWilliams JR** (1968) Studies on a maize mutant sensitive to low temperature. I. Influence of temperature and light on the production of chloroplast pigments. Plant Physiol **43:** 1967–1972
13. **Nelson OE** (1967) Biochemical genetics of higher plants. Annu Rev Genet **1:** 245–268
14. **Porra RJ, Thompson WA, Kreidemann PE** (1989) Determination of accurate extinction coefficients and simultaneous equations for assaying chlorophylls *a* and *b* extracted with four different solvents: verification of the concentration of chlorophyll standards by atomic absorption spectroscopy. Biochim Biophys Acta **975:** 384–394
15. **Ronnenkamp RR, Gorz HJ, Haskins FA** (1975) Genetic studies of induced mutants in *Melilotus alba*. IV. Inheritance and complementation of six additional chlorophyll-deficient mutants. Crop Sci **15:** 187–188
16. **Smillie RM, Nielsen NC, Henningsen KW, von Wettstein D** (1977) Development of photochemical activity in chloroplast membranes. I. Studies with mutants of barley grown under a single environment. Aust J Plant Physiol **4:** 415–438
17. **Somerville CR** (1986) Analysis of photosynthesis with mutants of higher plants and algae. Annu Rev Plant Physiol **37:** 467–507
18. **Somerville CR, Ogren WL** (1982) Isolation of photorespiration mutants in *Arabidopsis thaliana*. *In* M Edelman, RB Hallick, N-H Chua, eds, Methods in Chloroplast Molecular Biology. Elsevier Biomedical Press, Amsterdam, pp 129–138
19. **Specht JE, Haskins FA, Gorz HJ** (1975) Contents of chlorophyll *a* and *b* in chlorophyll-deficient mutants of sweetclover. Crop Sci **15:** 851–853
20. **Yang C-M, Osterman JC, Markwell J** (1990) Temperature sensitivity as a general phenomenon in a collection of chlorophyll-deficient mutants of sweetclover (*Melilotus alba*). Biochem Genet **28:** 31–40